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APPLICATION NUMBER: *60/448,468*
FILING DATE: *February 21, 2003*

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

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Atty. Dkt. No. 024569-0109

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Ernest LOUMAYE

Title: A NEW UTILISATION FOR GNRH AGONISTS

Appl. No.: Unknown

Filing Date: 02/21/2003

Examiner: Unknown

Art Unit: Unknown

PROVISIONAL PATENT APPLICATION TRANSMITTAL

Commissioner for Patents
Box PROVISIONAL PATENT APPLICATION
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Sir:

Transmitted herewith for filing under 37 C.F.R. § 1.53(c) is the provisional patent application of:

Ernest LOUMAY
Massongy, France

[X] Applicant claims small entity status under 37 CFR 1.27(c)(1).

Enclosed are:

[X] Application Data Sheet (37 CFR 1.76) (2 pages).

[X] Specification, Claim(s), and Abstract (26 pages).

Atty. Dkt. No. 024569-0109

The filing fee is calculated below:

		Rate		Fee Totals
Basic Fee		$160.00		$160.00
[X]	Small Entity Fees Apply (subtract ½ of above):		=	$80.00
	TOTAL FILING FEE:		=	$80.00

[X] A check in the amount of $80.00 to cover the filing fee is enclosed.

[X] The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Assistant Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

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Respectfully submitted,

Date February 21, 2003

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Application Data Sheet

Application Information

Application Type::	Provisional
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Computer Readable Form (CRF)?::	No
Title::	A NEW UTILISATION FOR GNRH AGONISTS
Attorney Docket Number::	024569-0109
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawing Sheets::	0
Small Entity?::	Yes
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

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Representative Information

Representative Customer Number::	22428	

Domestic Priority Information

Application::	**Continuity Type::**	**Parent Application::**	**Parent Filing Date::**

Foreign Priority Information

Country::	**Application number::**	**Filing Date::**	**Priority Claimed::**

Assignee Information

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Title: A NEW UTILISATION FOR GnRH AGONISTS

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The present invention concerns the use of an agonist of an hypothalamic hormone for the preparation of a pharmaceutical agent to support the luteal phase during the infertility treatment of female mammals and more specifically of women.

The menstrual cycle in woman is composed of three distinct phases (Yen S. and Jaffe R. 1986, Reproductive Endocrinology: Physiology, Pathophysiology and Clinical Management, W.B. Saunders Company, Philadelphia, PA, USA.):

1. The *follicular phase* during which several ovarian follicles are recruited, followed by the selection and the dominance of one follicle. This phase lasts approximately 12 days and is characterized by a progessive rise of serum estradiol levels and low progesterone levels. This phase is the result of the secretion of a hormone call FSH ("Follicle Stimulating Hormone") by the anterior pituitary gland.

2. A *peri-ovulatory* phase (also called *ovulation*) which lasts approximately 48 hours and is characterized by a sudden rise in serum LH ("Luteinizing Hormone", another hormone secreted by the pituitary gland). This phase ends when the "*corpus luteum*" is formed. This phase includes the following sequence of events:

- *The final follicular maturation:*
  - The follicle steroidogenesis switches from a preferential secretion of estradiol to a preferential secretion of progesterone.
  - The oocyte resumes its meiosis.
- *The ovulation:*
  - The follicle is ruptured and the oocyte leaves the ovary
- *The corpus luteum formation*
  - The empty follicle is re-organized to form the corpus luteum.

3) The *luteal phase* during which the corpus luteum secretes large amount of progesterone as well as somewhat smaller quantities of 17 OH-progesterone, estradiol, estrone and relaxin. This phase lasts approximately 14 days and depends of an appropriate secretion of LH by the pituitary gland.

During the luteal phase, progesterone works together with estradiol on endometrial cells to provide an environment favorable for the embryo implantation.

The luteal phase can be deficient. This can result from a deficit in progesterone secretion by the corpus luteum. In this case, serum progesterone levels are below 10 ng/ml. The luteal deficiency can also result from a shortening of the luteal phase (i.e. less than 11 days). The clinical consequences of a luteal phase deficiency are failure of embryo implantation, or a miscarriage if the pregnancy has already started (Yen S. and Jaffe R. 1986, Reproductive Endocrinology: Physiology, Pathophysiology and Clinical Management, W.B. Saunders Company, Philadelphia, PA, USA.).

A luteal phase deficiency is a common characteristic of cycles during which follicular development is stimulated with pharmacological agents for the treatment of infertility. This has been reported both in anovulatory patients undergoing ovulation induction therapy, and in ovulatory patients undergoing stimulation of multiple follicular development prior to intra-uterine insemination (IUI) or prior to Assisted Reproductive Technics (ART) such as *in vitro* fertilisation (IVF) and intra-cytoplasmic insemination (ICSI).

The luteal phase deficiency has been observed in cycles stimulated with clomiphene citrate, FSH/hMG, FSH/hMG with a GnRH agonist pre-treatment ("Gonadotrophin releasing hormone") as well as FSH/hMG with a GnRH antagonist co-treatment (Beckers NG *et al.* 2002, Comparison of non-supplemented luteal phase characteristics following the administration of r-hCG, r-hLH or GnRH agonist to induce final oocyte maturation in in vitro fertilisation patients. Hum. Reprod. 17: Abstract Book 1; O-157.; Pritts E.A. and Atwood A.K. 2002, Luteal support in infertility treatment: a meta-analysis of the randomized trials. Hum Reprod 17: 2287-2299). The luteal phase deficiency is mainly attributed to the elevated serum estradiol levels resulting from the pharmacological stimulation of the ovaries.

The pharmacological support of the luteal phase, also called "luteal supplementation", is mandatory in infertility treatments in order to significantly increase the embryo implantation rate, the pregnancy rate and to reduce the miscarriage rate (Pritts E.A. and Atwood A.K. 2002, Luteal support in infertility treatment: a meta-analysis of the randomized trials. Hum Reprod 17: 2287-2299).

Two drugs are routinely used for the luteal support. The first is natural progesterone, the second is the human chorionic gonadotropin (hCG). Progesterone is administered intra-muscularly (IM) or vaginally. The therapeutic objective is to increase serum progesterone levels. HCG is administered intra-muscularly (IM) or sub-cutaneously (SC). HCG is a naturally occurring agonist of LH and therefore it stimulates progesterone secretion by the corpus luteum.

In ART treatments, a luteal support by hCG or progesterone IM significantly increases pregnancy rate (Pritts E.A. and Atwood A.K. 2002, Luteal support in infertility treatment: a meta-analysis of the randomized trials. Hum Reprod 17: 2287-2299). The odds to obtain a pregnancy with hCG compared to no luteal support is 2.72 (CI: 1.56-4.90; $p<0.05$) and with progestérone IM is 2.38 (CI: 1.36-4.27; $p < 0.05$). Progesterone administered by vaginal route, although superior to no luteal support, is not as effective as progesterone IM. With vaginal progesterone the odds to obtain a pregnancy is 2.11 with a C.I. of 0.95-4.67 (NS). Furthermore, the relative efficacy of IM progesterone vs vaginal progesterone is 1.33 with a C.I. of 1.02 -1.75, in favor of the IM route ($p<0.05$) (Pritts E.A. and Atwood A.K. 2002, Luteal support in infertility treatment: a meta-analysis of the randomized trials. Hum Reprod 17: 2287-2299).

The drawbacks of IM progesterone are: (i) the injections must be performed daily for more than two weeks, (ii) the progesterone solution is oily and therefore injections are painful, (iii) these injections can trigger an inflammatory reaction and even a sterile abcess, (iv) IM injections are not easy for self-administration by the patient therefore often requiring paramedical assistance.

The drawbacks of hCG as luteal support are: (i) its use is associated with a rare but potentially life-threatening adverse event called ovarian hyperstimulation syndrome (OHSS), (ii) it must be injected, (iii) it induces a false positive pregnancy test, delaying the pregnancy diagnostics, (iv) it is a biological product extracted from urine or from culture media containing animal sera, and therefore presents a, at least theoritical risk, of contamination by infectious particles (e.g. viruses ou prions)(Reichl H *et al.*, 2002 Prion transmission in blood and urines: what are the implications for recombinant and urinary-derived gonadotropins ? Hum Reprod 17: 2501-2508). For all these reasons many doctors refrain of using hCG as luteal support.

The present invention aims at avoiding all these drawbacks by the use of an agonist of an hypothalamic hormone, i.e. an agonist of GnRH, for the preparation of a pharmaceutical preparation to support the luteal phase during the infertility treatment of female mammals and more specifically of woman.

According to this invention, the therapeutic agent is suitable to support the luteal phase after a spontaneous ovulation or after stimulation of follicular growth, triggering final follicular maturation and ovulation with one or several therapeutic agents.

In the detailed description of the invention application that follows, the following terms correspond to the following definitions:

The term "*Administration*" means to give a medication to a patient

The term "*Follicle*" refers to a structure in the ovary that contains and nurtures the oocyte. The oocyte is the female gamete or the female germinal cell.

In its final phase of development, the follicle becomes antral. This means that it has a cavity filled with fluid. At this stage of development, follicle growth is dependant from the pituitary FSH secretion. Follicle growth can be followed by measuring the cavity diameter with an ultrasound device. Typically, a pre-ovulatory follicle diameter measures between 16 and 24 mm (Balasch J. 2001. Inducing follicular development in anovulatory patients and normally ovulating women: current concepts and the role of recombinant gonadotropins. In Textbook of Assisted Reproductive Techniques eds D.K. Gardner, A. Weissman, C.M. Howles, Z. Shoham. Martin Dunitz 2001 pp 425-446).

"*Cumulus-oocyte complex*" refers to an oocyte surrounded by a mucinous matrix. The oocyte is freed of the cumulus after ovulation, during fertilisation. This occurs mainly thanks to an enzyme called hyaluronidase which is secreted by spermatozoa.

The term "*Peri-Ovulatory phase*" includes the events resulting from the sharp increase in serum LH at mid-cycle:

- "*Final follicular maturation*" refers to the biochemical and biological modifications occurring in the follicle and in the oocyte-cumulus complex during the mid-cycle LH rise but before the follicle rupture and the oocyte release. Briefly, these modifications include: (i) a change in granulosa cell steroidogenesis which switches from a mainly estradiol secretion towards a mainly progesterone

secretion, (ii) the resumption of the oocyte meiosis which transforms the oocyte from a germinal vesicle stage into a metaphase II stage.

- "*Ovulation*" refers to the process by which the oocyte leaves the ovary. First, the follicle makes protrusion at the surface of the ovary, it then ruptures and the oocyte-cumulus complex is expulsed with the follicular fluid.
- "*Corpus Luteum Formation*" refers to the re-organisation of the empty follicle after the oocyte-cumulus complex expulsion. Granulosa cells and theca cells (the two main cell populations of the follicle) undergo luteinization and neo-vascularisation to become a progesterone-secreting organ.

It is noteworthy that in medical jargon the wording "ovulation" is often used to describe both the peri-ovulatory events and the follicular rupture itself.

The term "*Luteal Phase*" refers to the lifespan of the corpus luteum during a spontaneous cycle without conception. Its lifespan is on average 14 days. The luteal phase begins the day after the mid-cycle rise in LH and finishes the day before the first day of menstruation.

The term "*Luteal Support*" defines the therapeutic interventions during the luteal phase aiming at supplementing or substituting the corpus luteal function for improving the embryo implantation and the early pregnancy development. Currently, two therapeutic agents are used for luteal support i.e. hCG and natural progesterone.

The term "*Assisted Reproductive Technics*" (ART) refers to medical interventions aiming at obtaining a pregnancy. These methods imply, by definition, manipulation of the male gametes (the spermatozoa) and/or the female gamete (the oocyte). In the most often used ART methods, follicular growth is first stimulated with one or several pharmaceutical agents. This is followed by methods for facilitating fertilization such as intra-uterine insemination (IUI) or *in vitro* fertilization (IVF). IUI consists in introducing a spermatozoa suspension into the uterine cavity using a fine catheter. IVF consists first to retrieve the oocytes from the ovary using a transvaginal echoguided aspiration needle. Then the oocytes are co-incubated with spermatozoas *in vitro* for obtaining a natural fertilization.

ICSI, which is a variation of the IVF method, is identical to IVF except that the fertilization is obtained by micro-injecting one spermatozoa directly in the oocyte

cytoplasm. The embryos resulting from IVF and ICSI are maintained in culture medium during a few days before being replaced in the patient's uterus or to be frozen for subsequent replacement.

The term "*Gonadotrophin releasing hormone (GnRH)*" refers to a peptidic hormone secreted by a specific area of the brain called hypothalamus. This decapeptide plays a pivotal role in the mechanisms of reproduction in many species and specifically in humans. GnRH acts on a specific cell population in the anterior pituitary gland where it bounds to a specific membrane receptor. It activates this receptor provoking an immediate secretion of LH and FSH in the blood stream.

The term *"GnRH agonist"* refers to synthetic or natural analogs of the native GnRH which have the capacity to recognise and activate GnRH receptors. These analogs may be peptide or non-peptidic molecules (peptido-mimetics).

The term "*Follicle Stimulating Hormone (FSH)* " refers to a pituitary hormone which stimulates ovarian follicle growth. FSH is part of a hormone family called the gonadotropins. Human FSH therapeutic preparations are obtained by extraction from biological fluids reach in FSH such as the urine of postmenopausal women. FSH can also be extracted from culture medium in which genetically modified cells produce human FSH (e.g. DNA recombination of CHO cells; Loumaye E., Howles C. 1999 Superovulation of Assisted Conception: The new Gonadotrophins. In Textbook of In Vitro Fertilization and Assisted Reproduction. P. Brinsden Eds, Parthenon Publishing). For the present invention the term FSH refers to a mix of several FSH isoforms, as well as to one specific FSH isoform which can be naturally occurring or obtained by a technical process. The term FSH also refers to hybrid molecules or chimeric molecules, to peptides and peptidomimetics which display FSH activity either by activation of the FSH receptor or by biochemical interaction at the post-receptor level in FSH target cells.

The term FSH also includes therapeutic preparations such as hMG (*human menopausal gonadotrophins*) or recombinant FSH preparations in which small amount of LH and/or hCG are added.

The term "*Selective estrogen receptors modulators (SERM)* " refers to all chemical or polypeptidic compound which acts totally or partially as activator of the oestrogen receptors, in particular at hypothalamic and pituitary lelevs. Exemples of SERM include clomiphene, tamoxifene, and raloxifene.

The term "*Aromatase inhibitor*" refers to all chemical, steroidal, and polypeptidic compounds which block the activity of an enzyme called aromatase. This enzyme catalyses the conversion of androgens into oestrogens. Exemples of aromatase inhibitors include anastrozole, letrozole and exemestane.

The term "*Phosphodiesterase Inhibitors*" refers to all chemical compounds which block or inhibit phosphodiesterases. Phosphodiesterases are enzymes inactivating cycliques nucleotides such as cyclic AMP and cyclic GMP. Inhibition of this activity results in the accumulation of these cyclic nucleotides prolonging in the target tissue, the signal induced by FSH ou LH. An exemple of phosphodiesterase inhibitor is theophylin.

GnRH is a neuropeptide which stimulates LH and FSH secretion by the pituitary gland. These two hormones are essential for the menstrual cycle. Analogs derived from native GnRH structure have been synthezised. These analogs often present agonist activity that is enhanced compared to the native peptide. This increased activity is mainly due to an enhance resistance to degradation and a higher affinity for the pituitary GnRH receptor.

In clinics, these GnRH agonists are used for reducing LH and FSH secretion by a desensitization mechanism of the pituitary cells. The main therapeutic indications of these agonists are prostate cancer, endometriosis, and the prevention of premature rise of LH during stimulation of follicular development prior to ART (Loumaye E. 1990 The control of endogenous secretion of LH by gonadotrophin-releasing hormone agonists during ovarian hyperstimulation for in-vitro fertilization and embryo transfer. Hum Reprod. 5:357-76). Contrasting with the common use of GnRH agonists to inhibit LH and FSH secretion, the therapeutic use of their agonist property (to stimulate the LH and FSH secretion) has been very limited up to know. In infertility treatments, the ability of these substances to stimulate LH secretion has been used to trigger ovulation at mid-cycle (Lanzone, A *et al.*, 1989 LH surge induction by GnRH agonist at the time of ovulation.

Gynecol Endocrinol 3: 213-220; Buckett W.M. *et al.*, 1998, Induction of the endogenous gonadotrophin surge for oocyte maturation with intra-nasal GnRH analogue (buserelin): effective minimal dose. Hum Reprod 13: 811-814, 1998; Fauser BC *et al.*, 2002, Endocrine profile after triggering of final oocyte maturation with GnRH agonist after co-treatment with the GnRH antagonist Ganirelix during ovarian hyperstimulation for *in vitro* fertilisation. J Clin Endocrinol Metab 87: 709-715). However, in this case, the luteal phase was also found to be deficient and the pregnancy rate was low (Fauser BC *et al.*, 2002, Endocrine profile after triggering of final oocyte maturation with GnRH agonist after co-treatment with the GnRH antagonist Ganirelix during ovarian hyperstimulation for *in vitro* fertilisation. J Clin Endocrinol Metab 87: 709-715; Beckers *et al.*, 2002, Comparison of non-supplemented luteal phase characteristics following the administration of r-hCG, r-hLH or GnRH agonist to induce final oocyte maturation in in vitro fertilisation patients. Hum. Reprod. 17: Abstract Book 1; O-157).

Only one attempt to use GnRH agonist to support the luteal phase has been reported in the medical litterature (Schmidt-Sarosi C. *et al.*, 1995, Ovulation triggering in clomiphene citrate-stimulated cycles: human chorionic gonadotropin versus a gonadotropin releasing hormone agonist. J Ass. Reprod. & Genetics. 12: 167-174). This attempt however failed as indicated in the report by the abnormaly low serum progesterone levels, a deficit in progesterone at the endometrium level, and low pregnancy rate.

It is noteworthy that a reduction in progesterone levels (via a reduction in lH levels) has also been reported after repeated administration of a GnRH agonist during the luteal phase of spontaneous cycles (Lemay *et al.*, 1982, Sensitivity of pituitary and corpus luteum responses to single intranasal administration of buserelin in normal women. Fertil Steril 37: 193-200 ; Lemay *et al.*, 1983, Gonadotroph and corpus luteum responses to two successive intranasal doses of a luteinising hormone-releasing hormone

agonist at different days after the mid-cycle luteinising hormone surge, Fertil Steril 39: 661-887).

All currently available evidences therefore point toward a negative effect of GnRH agonists on the luteal function and in any case no support effect. Therefore, up to date, GnRH agonists are considered by "l'homme de l'art" and are essentially used as therapeutic agent to inhibit LH and FSH secretion through a desensitization mechanism , rather than to stimulate their secretion.

However, present invention results specifically from the observation that very surprisingly, the use of a GnRH agonist in the preparation of a pharmaceutical agent for luteal support is fully possible and brings significant advantages when compared to agents currently used in this indication.

GnRH agonists can be used for luteal support either after a spontaneous ovulation, or after stimulation of follicular growth and induction of final follicular maturation and ovulation with one or several therapeutic agents. In the latter case, the therapeutic agent triggering final follicular maturation and ovulation can also be selected among GnRH agonists; another agonist or preferably the same agonist that the one used to support the luteal phase.

In some circumstances, a premature LH rise may occur during the follicle growth phase. This has a deleterious effect on the oocyte viability and can even trigger a premature ovulation, resulting in the treatment cycle cancellation. In order to prevent such premature LH rise, one can use the therapeutic agent suitable for luteal phase support after the administration of a GnRH antagonist which is administered during the last days of follicle growth stimulation, typically as soon as follicles reached a mean diameter around 12 and 14 mm.

If most patients have an adequate luteal support when receiving a GnRH agonist according to the present invention, a minority may still have low serum progesterone levels, e.g. less than 10 ng/ml or a short luteal phase, e.g.g less than 11 days. In this case, it is recommended to add to the GnRH agonist from the present invention, another luteal support such as natural progesterone, or a progestagen, or hCG, or LH, or one or more isoform of LH or of hCG, or a peptidomimetic of LH or of hCG, or an LH or an hCG analog with a modified pharmacokinetic, or a phospodiesterase inhibitor or a combination of two or more of these agents.

The combination of one or more of these therapeutic agents will however be done at lower doses than those used when the agent is used alone to support the luteal phase.

According to a specific application of the present invention, follicular growth is stimulated with a folliculo-stimulating agent starting at the beginning of a spontaneous cycle or after induction of menstruation with a contraceptive pill or a progestagen. Agents stimulating follicular growth will be selected among hMG, urine-derived FSH, recombinant FSH, one or more FSH isoforms, FSH mimetics, FSH analog with a modified pharmacokinetic (e.g. chimeric molecules), SERM, aromatase inhibitors, phosphodiesterase inhibitors, or a combination of two or more of these agents.

For exemple, SERM are selected among clomiphene, tamoxifene, or raloxifene or a combination of two or more of these agents, while aromatase inhibitors can be selected among anastrozole, letrozole or exemestane or a combination of two or more of these agents.

Equally, according to the application of this invention, the use of a phosphodiesterase inhibitor such as theophylin, as agent stimulating follicular growth will allow to prolong the ovarian effect of endogenous and/or exogenous FSH by preventing the catabolism (i.e. the destruction) of FSH second messenger, namely cyclic AMP.

This stimulation is followed by triggering final follicular maturation and ovulation with one or more of the following agents: hCG, or LH, or one or more isoform of LH or of hCG, or a peptidomimetic of LH or of hCG, or an LH or an hCG analog with a modified pharmacokinetic, or a phospodiesterase inhibitor or a combination of two or more of these agents.

According to another application of the present invention, follicular growth stimulation and induction of ovulation is followed by an IUI or an oocyte recovery procedure. The oocytes will be used for *in vitro* maturation, *in vitro* fertilization for subsequent uterus transfer.

All insemination methods are acceptable and the selection of one method is a medical decision. Sexual intercourses and IUI are usually recommended the day after triggering ovulation, and will eventually be repeated the day after. For IVF and ICSI, oocyte recovery must be done within a very precise timing, i.e. 34 to 40 hours after triggering final follicular maturation. For recovering the oocytes, the follicular fluid is aspirated using a needle guided with ultrasound. The aspirated fluid is examined under a binocular microscope for identifying cumulus-oocyte complexes. Those complexes are transferred in an appropriate culture medium and kept in an incubator maintaining well defined and suitable temperature, humidity and gaz conditions.

According to the present invention, the luteal support provided by a GnRH agonist may be associated with a therapeutic agent involved in the embryo implantation. Indeed, if an embryo implantation requires an endometrium prepared with an adequate luteal support, it is also known that other factors such as cytokines play a critical role in this process (Lessey BA.. The role of the endometrium during embryo implantation. Hum Reprod 2000: 15 Suppl 6:39-50). A specific exemple is the critical role played by Leukemia Inhibitory Factor (LIF) in embryo implantation (Stewart CL *et al.* Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 1992;359:76-9). In patients having implantation problems related to a relative or absolute LIF deficiency, it is recommended to add to the GnRH agonist for luteal support as described in the present invention, native or natural LIF, or recombinant LIF, or a peptidic or a non-peptidic LIF analog and/or another cytokine involved in embryo implantation mechanisms.

The therapeutic agent containing the GnRH agonist used to support the luteal phase for infertility treatment of a female mammals, more specifically of woman, may be administered to the patient either by the nasal route, or the oral route, or the subcutaneous route, or the intramuscular route, or the vaginal route, or the rectal route, or the transdermal route or the lung route.

The GnRH agonist used in the present invention may be selected among a native or natural GnRH from mammals or any other animal species, or a recombinant, or a synthetic peptide agonist of GnRH, or a non-peptidic agonist of GnRH, or a chimeric molecule of GnRH. The latter molecule may include a functional portion, peptidic or non-peptidic, of

GnRH and will be obtained by molecular biology methods known by a man skilled in the art. More specifically, the GnRH agonist will be selected among a group of substances which includes buserelin, nafarelin, triptorelin, leuprorelin ou gosserelin, and analogs with derived structures having essentially a GnRH activity, a combination of two or more of these agonists.

The therapeutic agent containing the GnRH agonist used to support the luteal phase for infertility treatment, will typically be administered within the first three days following ovulation trigger up to the moment a pregnancy is well established. Preferably, the administration will be started as soon as the first day following ovulation trigger. The dose of agonist is variable and will depend essentially of the agonist used, its pharmacokinetic and pharmacodynamic characteristics, as well as its mode of administration.

According to this invention, and preferably, the GnRH agonist used will be buserelin, the preferred route of administration will be intra-nasal, at one or several daily doses between 50 and 400 µg, preferably 100 µg.

The GnRH agonist administration frequency is also critical and must be defined for each agonist in regards of its pharmacokinetic and pharmacodynamic properties as well as its formulation. According to the preferred use, the therapeutic agent containing buserelin to be used for supporting the luteal phase during treatment of infertility, will be administered at a frequency between two times a day (on average at 12 hours interval) and one administration every three days, but preferentially one administration every two days (approximately once every 48 hours).

The total period of administration of the GnRH agonist must cover at least the embryo pre-, peri- and early post-implantation period. After implantation, the embryo will indeed progressively secure its own luteal support through hCG secretion by trophoblast cells. Practically this means a duration of administration for the agonist between 7 and 28 days. Preferably, the total duration of buserelin administration will be 14 days.

The stimulation of follicular growth with FSH or derived compounds must last on average 10 days. The ovarian response to the stimulation is monitored by measuring, with ultrasound, the number and the diameter of all growing follicles. An additional method for

this monitoring is to measure serum oestradiol levels (Shoham, 2001, Drug used for controlled ovarian stimulation: clomiphene citrate and gonadotropins. In *Textbook of Assisted Reproductive Techniques* eds D.K. Gardner, A. Weissman, C.M. Howles, Z. Shoham. Martin Dunitz 2001.pp 413-424 ; Balasch, 2001, Inducing follicular development in anovulatory patients and normally ovulating women: current concepts and the role of recombinant gonadotropins. In Textbook of Assisted Reproductive Techniques eds D.K. Gardner, A. Weissman, C.M. Howles, Z. Shoham. Martin Dunitz 2001 pp 425-446).

For agents stimulating endogenous FSH, such as SERM and aromatase inhibitors, they are usually administered by oral route for a period between one and seven days starting at the beginning of a menstrual cycle (Fisher *et al.* 2002, A randomized double-blind comparison of the effetcts of clomiphene citrate and the aromatase inhibitor letrozole on ovulatory function in normal women. Fertil Steril 78: 280-285.).

A third possibility is to use phosphodiesterase inhibitors that will increase and prolong the ovarian effect of endogenous and/or exogenous FSH by preventing the catabolism (inactivation and destruction) of FSH second messenger i.e. cyclic AMP.

When a GnRH agonist is selected for triggering final follicular maturation, it will preferably, be the same that the agonist used to support the luteal phase. According to the present invention, it is preferred to use buserelin as GnRH agonist and the preferred mode of administration will be a single intra-nasal administration, at a dose between 50 and 600 µg, the preferred dose being 200 µg.

One embodiment of the present invention also forsees a pharmaceutical preparation suitable for delayed and controlled release of the agonist as defined in the present
invention. The GnRH agonist can, for exemple, be incorporated in a matrix of biocompatible polymer allowing delayed and controlled release. All biocompatible polymers, well known by the man skilled in the art are potential candidate to be used in this invention.

One additional aspect of the present invention is to provide a tool for treatment commonly called "kit", which will include one or several therapeutic agents to trigger final follicular maturation, ovulation and the GnRH agonist to support the luteal phase. Preferrably, the therapeutic agent used to trigger final follicular maturation and ovulation will be the same as the agonist used to support the luteal phase. According to the present invention, the GnRH agonist is buserelin. Preferably, the therapeutic agent used for triggering final follicular maturation and ovulation, as well as the agonist used for luteal support will be formulated in dosage and unit, or multiple units, sufficient for one to three, but preferably one cycle of treatment. The formulated product may be included in a packaging or an administration device easing the GnRH agonist administration to the patient.

Another aspect of the present invention is a method for treating infertility using GnRH agonists to support the luteal phase. This method includes the following phases:

a) stimulation of follicle growth phase, final maturation of follicles, ovulation with one or several therapeutic agents,

b) use of a pharmaceutical agent such as a GnRH agonist to support the luteal phase as defined above in the description of the various ways to apply the invention.

The present invention as well as the various ways to apply it are illustrated by the following exemples which are not limiting:

Exemple 1

Patients suffering from unexplained infertility, or from infertility resulting from mild to moderate endometrioisis, or an infertility resulting from a mild or moderate alteration of their partner sperm, and whom have not conceived despite regular sexual intercourses during a period of one or two years, usually undergo medical assistance. The most often used treatment is ovarian stimulation coupled to an IUI (Hughes G.H. 1997, The effectiveness of ovulation induction and intra-uterine insemination in the treatment of persistent infertility: a meta-analysis. Hum Reprod. 12: 1865-1872). This treatment may be preceeded by an oral contraceptive pill to induce menstruation and better plan the

treatment cycle. On day 2 to 5 of menstruations, the patient will start taking either clomiphene citrate, or an aromatase inhibitor or FSH. Clomiphene citrate will be administered orally, once a day, from day 3 to day 7 at a dose of 50 to 250 mg/day. Letrozol, an aromatase inhibitor will be taken orally for 1 to 7 days, at a daily dose of 0.5 to 10 mg/day. FSH will be administered subcutaneously or intramuscularly from day 2 to 3 of menstruations, for 5 to 12 days, at a daily dose of 37.5 to 150 IU/day.

Whatever the therapeutic agent used to stimulate follicular growth, the ovarian response to treatment will be followed on a regular basis using transvaginal ultrasound and by measuring serum oestradiol levels. Typically, a first assessment will be performed on day 6 of the stimulation. The number and the size of each follicle will be recorded.

Estradiol serum concentration will be measured using a suitable and validated assay, most often a RIA, IRMA or ELISA.

When at least one, and maximum three follicles reach a mean diameter of 16 mm or more, and that serum estradiol level does not indicate a significant risk of OHSS (i.e. serum estradiol < 1500 pg/ml), the patient will receive one administration of GnRH agonist to trigger ovulation. For that purpose the dose will be between 50 and 600 µg of buserelin administered intra-nasally, the preferred dose being 200 µg.

Between 12 and 48 hours after triggering ovulation, the patient's partner will provide a sperm sample, preferrably after 2 to 5 days of abstinence. Motile sperm cells will be separated from seminal fluid, dead sperm cells, leucocytes, and cellular debris using a suitable method such as a "swim-up" or a Percol gradient. On that same day, the motile sperm cell suspension will be used to perform an intra-uterine insemination with a catheter inserted into the uterine cavity via the cervix. The procedure may be repeated on the next day.

From that day and for a period of 14 days, the patient will receive between three times a day and once every three days (preferably once every two days), one intra-nasal administration of 50 to 400 µg of buserelin, preferably 100 µg. These buserelin administrations will be self-administered by the patient. These intra-nasal administrations

take less than one minute, necessitate no preparation and no material, and do not carry the risk of adverse local reactions such as pain and abces.

Exemple 2

Patients from exemple 1 who would not be pregnant after 3 to 6 cycles of IUI, will be proposed a treatment with IVF or ICSI. These treatments will be proposed upfront to patients whom infertility cause is a severe tubal problem, or a severe endometriosis or a partner's severe sperm severe alteration. The patient can be treated prior her IVF or ICSI cycle with a oral contraceptive to precisely program her menstruation date.
This is done to better manage the couple and the ART center agenda. When menstruations occur, an ovarian stimulation will be performed with FSH at a dose between 75 and 600 IU per day based on the patient's characteristics (e.g.: age, ovarian reserve status, weight, etc....). This treatment can be done with FSH alone, but may also include clomiphene citrate or an aromatase inhibitor. Five to 7 days after the beginning of the stimulation, or when the leading follicles reach a mean diameter of 14 mm, a GnRH antagonist will be administered to prevent a premature LH rise in the blood. Follicular growth will be monitored using vaginal ultrasound and by measuring serum estradiol levels. These controls will be performed every 2 to 3 days.
When at least two follicles have reached a mean diameter of 16 mm or more, when the total number of follicles does not exceed 25 to 30, and when serum estradiol concentration does not indicate a risk for ovarian hyperstimulation (serum estradiol level < 4000 pg/ml), the patient will receive one administration of GnRH agonist to trigger final follicular maturation. For that purpose, she will receive between 50 and 600 µg of busereline intra-nasaly, the preferred dose being 200 µg. The timing of buserelin administration will be very precise since it will determine the timing of oocyte retrieval procedure which must be done between 35 and 38 hours after the trigger.

On the second day after GnRH administration, the patient will be admitted in the ART clinical unit for the oocyte retrieval procedure. Oocytes will be retrieved by echoguided transvaginal aspiration of the follicles. This procedure is performed under light anesthesia. Aspirated follicular fluids will be examined under binocular microscope to identify oocyte-cumulus complexes. These complexes will be transferred in an appropriate culture

medium, and maintain in an incubator in adequate temperature, gaz and humidity conditions. Several hours after retrieval, oocytes will be inseminated either by co-incubation in a motile sperm cell suspension, or by direct injection of one sperm cell in the oocyte cytoplasm using a micro-manipulator. The patient will leave the ART center on that same day.

On the day preceeding the oocyte retrieval procedure, and for a period of 14 days, the patient will receive between 3 times a day and once every three days (preferably once every two days), one intra-nasal administration of 50 to 400 µg of buserelin, preferably 100 µg. These intra-nasal administrations take less than one minute, necessitate no preparation and no material, and do not carry the risk of adverse local reactions such as pain and abces.

The day after insemination, fertilisation of the oocytes will be assessed by visualizing under a microscope the presence of two pronuclei in the cytoplasm. The first cleavage stage of the embryos will be observed within the next 48 houres. The embryo transfer into the uterus will usually be performed on day 3 after oocyte retrieval or later if the embryo culture is pursued up to the blastocyst stage. Most often, two embryos are replaced if the patient is less than 35 years old, and three are replaced if the patient is more than 35 years old.

On day 13, after final follicular maturation trigger, a pregnancy test will be performed by measuring hCG concentration in the patient's urines or blood (serum). A positive hCG test unquestionably will mean that the patient is pregnant since she would not have receive exogenous hCG.

Claims

1. Use of an agonist of an hypothalamic hormone for the preparation of a pharmaceutical agent for the infertility treatment of female mammals, wherein the agonist is a GnRH agonist and the pharmaceutical agent is suitable to be used for luteal phase support.

2. Use according to claim 1, wherein the pharmaceutical agent is suitable to be used for luteal phase support after a spontaneous ovulation.

3. Use according to claim 1, wherein the pharmaceutical agent is suitable to be used for luteal phase support after stimulation of follicular growth, induction of final follicular maturation and ovulation with one or more therapeutic agents.

4. Use according to claim 3, wherein the pharmaceutical agent triggering final follicular maturation and ovulation is a GnRH agonist.

5. Use according to claim 3 and 4, wherein the pharmaceutical agent triggering final follicular maturation and ovulation is the GnRH agonist used to support the luteal phase.

6. Use according to claim 3 and 4, wherein the pharmaceutical agent triggering final follicular maturation and ovulation is a GnRH agonist different from the GnRH agonist used to support the luteal phase.

7. Use according to claim 3, wherein the pharmaceutical agent suitable for luteal phase support is used after administration of a GnRH antagonist during the last days of follicular growth stimulation.

8. Use according to any of the preceeding claims, wherein the pharmaceutical agent suitable for luteal phase support is administered in combination with natural progesterone, a progestagen, hCG, an agonist analog of hCG, LH, an agonist analog of LH, or a non-peptidic modulator of cyclicAMP.

9. Use according to claim 1 to 7, wherein the pharmaceutical agent suitable for luteal phase support is administered in combination with a cytokine involved in the embryo implantation mechanisms.

10. Use according to claim 9, wherein the cytokine is native LIF, recombinant LIF, a peptidic or a non-peptidic agonist analog of LIF.

11. Use according to claim 1 to 10, wherein the stimulation with a therapeutic agent is followed, before ovulation, by an oocyte retrieval procedure.

12. Use according to claim 11, wherein oocytes are to undergo an *in vitro* maturation.

13. Use according to claim 11, wherein oocytes are to undergo an *in vitro* fertilization.

14. Use according to claims 1 to 10, wherein the stimulation with a therapeutic agent is followed, after ovulation trigger, with an insemination (IUI).

15. Use according to any of the preceeding claims, wherein the GnRH agonist route of administration is intra-nasal, oral, sub-cutaneous, intra-muscular, vaginal, rectal, transdermal, or pulmonary.

16. Use according to any of the preceeding claims, wherein the GnRH agonist is a natural or native GnRH, a recombinant GnRH, a synthetic peptide agonist of GnRH, a non-peptidic GnRH agonist, or a molecular chimera of GnRH.

17. Use according to claim 16, wherein the GnRH agonist is selected from the group including buserelin, nafarelin, triptorelin, leuprorelin and gosserelin or a combination of two or more of these agonists.

18. Use according to claim 3, wherein the pharmaceutical agent stimulating follicular growth is selected from the group including hMG, urine-derived FSH, recombinant FSH, one or several FSH isoforms, FSH mimetics, FSH derivates with a modified duration of activity, SERM, aromatases inhibitors, phosphodiesterase inhibitors, or a combination of two or more of these agents.

19. Use according to claim 18, wherein SERM are selected from the group including clomiphene citrate, tamoxifen, or raloxifen or a combination of two or more of these agents.

20. Use according to claim 18, wherein the aromatase inhibitor is selected from the group including anastrozol, letrozol or exemestane or a combination of two or more of these agents.

21. Use according to claim 3, wherein the pharmaceutical agent triggering final follicular maturation and ovulation is selected from the group including hCG, LH, one or more isoforms of hCG or LH, hCG and LH peptido-mimetics, hCG and LH derivates with a modified duration of activity, phosphodiesterase inhibitors or a combination of two or more of these agents.

22. Use according to claim 18 or 21, wherein the phosphodiesterase inhibitor is theophylin.

23. Use according to any of the preceeding claims, wherein the female mammal is a woman.

24. Use according to claim 17, wherein the GnRH agonist is buserelin.

25. Use according to claim 24, wherein buserelin administration is started within the first three days following ovulation trigger.

26. Use according to claim 25, wherein buserelin administration is started on the first day following ovulation trigger.

27. Use according to claim 24, 25, and 26 wherein buserelin is administered intra-nasally at a dose between 50 and 400 µg.

28. Use according to claim 27, wherein buserelin is administered intra-nasally at a dose of 100 µg.

29. Use according to claim 28, wherein buserelin is administered at a frequency between three times a day and once every three days.

30. Use according to claim 29, wherein buserelin is administered at a frequency of one administration every two days.

31. Use according to claim 29, wherein buserelin is administered during 7 to 28 days.

32. Use according to claim 29, wherein buserelin is administered during 14 days.

33. Use according to claims 4, 5 and 6, wherein the GnRH agonist is buserelin and is administered intra-nasally, once at a dose between 50 and 600 µg.

34. Use according to claim 33 wherein buserelin is administered intra-nasally at a dose of 200 µg.

35. Use according to claim 1, wherein the pharmaceutical agent is embedded in a biocompatible polymer matrix allowing sustained and controlled release.

36. Kit for the treatment of infertility in female mammals which includes:

- a GnRH agonist according to claim 1,
- one or more pharmaceutical agents to trigger final follicular maturation, and ovulation according to claims 4, 5, 6, 17 et 21.

37. Kit according to claim 36, wherein the pharmaceutical agent is the GnRH agonist used for the luteal support and wherein the GnRH agonist is formulated in dosage and unit required for one cycle of treatment.

38. Method of treatment for female mammals infertility, using a pharmaceutical agent which includes a GnRH agonist suitable for luteal phase support.

39. Method according to claim 38, wherein the pharmaceutical agent is suitable for use as luteal support after a spontaneous ovulation.

40. Method according to claim 38, wherein the pharmaceutical agent is suitable for use as luteal support after stimulation of follicular growth, trigger of final follicular maturation and ovulation, with one or more pharmaceutical agents.

41. Method according to claim 40, wherein the pharmaceutical agent used for triggering final follicular maturation and ovulation is a GnRH agonist.

42. Method according to claims 40 and 41, wherein the pharmaceutical agent used to trigger final follicular maturation and ovulation is the GnRH agonist used for the luteal phase support.

43. Method according to claims 40 and 41, wherein the pharmaceutical agent used to trigger final follicular maturation and ovulation is a GnRH agonist different from the GnRH agonist used for the luteal phase support.

44. Method according to claim 40, wherein the pharmaceutical agent suitable for luteal support is used after administration of a GnRH antagonist during the last days of follicular growth stimulation.

45. Method according to claims 38 to 44, wherein the pharmaceutical agent suitable for luteal phase support is administered in combination with natural progesterone, a progestagen, hCG, an agonist analog of hCG, LH, an agonist analog of LH, or a non-peptidic modulator of cyclicAMP.

46. Method according to claims 38 to 44, wherein the pharmaceutical agent suitable for luteal phase support is administered in combination with a cytokine involved in the embryo implantation mechanisms.

47. Method according to claim 46, wherein the cytokine is native or natural LIF, recombinant LIF, a peptidic or a non-peptidic agonist analog of LIF.

48. Method according to claims 38 to 47, wherein the stimulation with a therapeutic agent is followed, before ovulation, by an oocyte retrieval procedure.

49. Method according to claim 48, wherein oocytes are to undergo an *in vitro* maturation.

50. Method according to claim 48, wherein oocytes are to undergo an *in vitro* fertilization.

51. Method according to claims 38 to 50, wherein the GnRH agonist route of administration is intra-nasal, oral, sub-cutaneous, intra-muscular, vaginal, rectal, transdermal, or pulmonary.

52. Method according to claims 38 to 51, wherein the GnRH agonist is a natural or native GnRH, a recombinant GnRH, a synthetic peptide agonist of GnRH, a non-peptidic GnRH agonist, or a molecular chimera of GnRH.

53. Method according to claim 52, wherein the GnRH agonist is selected from the group including buserelin, nafarelin, triptorelin, leuprorelin and gosserelin or a combination of two or more of these agonists.

54. Method according to claim 40, wherein the pharmaceutical agent stimulating follicular growth is selected from the group including hMG, urine-derived FSH, recombinant FSH, one or several FSH isoforms, FSH mimetics, FSH derivates with a modified duration of activity, SERM, aromatases inhibitors, phosphodiesterase inhibitors, or a combination of two or more of these agents.

55. Method according to claim 54, wherein SERM are selected from the group including clomiphene citrate, tamoxifen, or raloxifen or a combination of two or more of these agents.

56. Method according to claim 54, wherein the aromatase inhibitor is selected from the group including anastrozole, letrozole or exemestane or a combination of two or more of these agents.

57. Method according to claim 40, wherein the pharmaceutical agent triggering final follicular maturation and ovulation is selected from the group including hCG, LH, one or more isoforms of hCG or LH, hCG and LH peptido-mimétics, hCG and LH derivates with a modified duration of activity, phosphodiesterase inhibitors or a combination of two or more of these agents.

58. Method according to claims 54 and 57, wherein the phosphodiesterase inhibitor is theophylin.

59. Method according to claim 38, wherein the female mammal is a woman.

60. Method according to claim 53, wherein the GnRH agonist is buserelin.

61. Method according to claim 60, wherein buserelin administration is started within the first three days following ovulation trigger.

62. Method according to claim 61,wherein buserelin administration is started on the first day following ovulation trigger.

63. Method according to claims 60, 61 and 62 wherein buserelin is used intra-nasally at a dose between 50 and 400 µg.

64. Method according to claim 63, wherein buserelin is used intra-nasally at a dose of 100 µg.

65. Method according to claims 60 to 64, wherein buserelin is used at a frequency between three times a day and once every three days.

66. Method according to claim 65, wherein buserelin is used at a frequency of one administration every two days.

67. Method according to claims 60 to 66, wherein buserelin is administered during 7 to 28 days.

68. Method according to claim 67, wherein buserelin is administered during 14 days.

69. Method according to claim 41, wherein the GnRH agonist is buserelin and is administered intra-nasally, once, at a dose between 50 and 600 µg.

70. Use according to claim 69, wherein buserelin is administered intra-nasally at a dose of 200 µg.

71. Use according to claim 38, wherein the pharmaceutical agent is embedded in a biocompatible polymer matrix allowing sustained and controlled release.

Abstract

The present invention concerns the use of an agonist of an hypothalamic hormone for the preparation of a pharmaceutical agent to support the luteal phase during infertility treatment of female mammals and more specifically of woman. According to this invention, the pharmaceutical agent is suitable to be used for supporting the luteal phase after a spontaneous ovulation or after stimulation of follicular growth, trigger of final follicular maturation and ovulation with one or several therapeutic agents.